Physical forces drive the movement of tissues within the early embryo. Classical and modern approaches have been used to infer and in rare cases measure mechanical properties and the location and magnitude of forces within embryos. Elongation of the dorsal axis is a critical event in early vertebrate development yet the mechanics of dorsal tissues in driving embryonic elongation that later support neural tube closure and formation of the central nervous system are not known. Among vertebrates, amphibian embryos allow complex physical manipulation of embryonic tissues that are required to measure the mechanical properties of tissues. We measure the stiffness of dorsal isolate explants of frog (*Xenopus laevis*) from gastrulation to neurulation. We find that tissues stiffen from less than 20 Pa to over 80 Pa in less than 8 h. Furthermore, by isolating specific axial and paraxial tissues we find that paraxial somitic mesoderm is nearly twice as stiff as either the notochord or neural plate and at least 20-fold stiffer than the endoderm. Dorsal isolates treated with acute acting cytoskeletal inhibitors or prepared from fibronectin knock-down embryos reveal that stiffness is the product of a contractile actomyosin cortex rather than a fibrillar fibronectin extracellular matrix. Increasing dorsal stiffness and spatial variation between germ layers during neurulation suggests a common vertebrate dorsal tissue architecture that may insure robust neural tube closure and provide positional cell identity prior to organogenesis.

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Program/Abstract # 157
Regulation of cytoarchitecture in development: The roles of IQGAP2, C-cadherin and Cdc42 in the early *Xenopus* embryos
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The control of cytoarchitecture is fundamentally important in multicellular organisms, both for tissue assembly during embryonic development and for adult tissue remodeling. The *Xenopus* blastula has proven to be a useful model system for studying the mechanisms of cytoarchitecture control during early development. Because cortical actin assembly at late blastula stage is controlled by mRNAs maternally transcribed and stored in the egg, depletion of individual stored mRNAs allows rapid loss of function, as well as epistatic experiments. We have previously shown that the cortical actin network is essential for rigidity and shape in the early embryos, and that the level of expression of C-cadherin on the cell surface is rate limiting for cortical actin assembly at the blastula stage. We have also identified two G protein-coupled receptors (GPCRs) that control cortical actin assembly by controlling the expression level of C-cadherin on the cell surface in the late *Xenopus* blastulae. However, it is unclear how the activity of C-cadherin is controlled at the cell surface to nucleate cortical actin assembly. Here, we provide evidence that cdc42, a member of small Rho GTPases, is essential to mediate C-cadherin controlled cortical actin assembly. IQGAP2, a multi-domain scaffold protein is identified as a major effector protein of Cdc42 that is necessary for cortical actin assembly controlled by C-cadherin at the cell surface. We also show that actin nucleation factor Arp2/3 complex is required for C-cadherin to assemble cortical actin at the cell surface.

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Program/Abstract # 158
A tale of two tails: Multiple pathways regulate cell adhesion and morphogenesis in the zebrafish tail
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Multiple signaling pathways regulate development of the posterior vertebrate body, which is derived from a population of progenitor cells called the tailbud. Fate specification and differentiation are closely linked with cell migration to ensure that, as some cells exit the tailbud and differentiate, other cells are retained in the tailbud as undifferentiated precursors to support later tail outgrowth.

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Program/Abstract # 159
Neuregulin-mediated ErbB3 signaling is required for DRG neuron formation
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ErbB3 is a receptor-type tyrosine kinase that has been shown to have important roles in neural and glial development. We found that *erbb3* mutant zebrafish have a defect in dorsal root ganglion (DRG) formation, as revealed early in development by absence of *neurogenin1*-positive nascent DRG neurons and later by absence of HuC/D-positive DRG neurons. To learn whether neural crest cell migration is affected in *erbb3* mutants, we followed neural crest migration in live transgenic embryos in which GFP expression is driven by the zebrafish *sox10* promoter. In *erbb3* mutants, overall motility of neural crest cells appeared unaffected. However, migrating neural crest cells failed to pause near the position where DRGs normally form. This is in contrast to wild types, in which a subset of migrating neural crest cells pause in this location for many hours. Transient blockade of ErbB receptors revealed that ErbB receptor signaling is required for DRG neuron formation around the time migrating neural crest cells pause. Together these results suggest that ErbB3 signaling is required for DRG progenitors to recognize their target position during migration. We isolated genes encoding the ErbB3 ligands Neuregulin 1 (Nrg1) and Neuregulin 2 (Nrg2) and found that zebrafish has one *nrg1* and two *nrg2* (*nrg2a* and *nrg2b*) genes. To learn whether any of the Nrgs are involved in DRG neuron formation, we knock them down individually and in combination using morpholinos and found that Nrg1 and Nrg2a act together during neural crest migration and DRG formation.

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Program/Abstract # 160
A role for zic genes during neural tube morphogenesis in zebrafish
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Our studies are aimed at understanding the conserved roles of Zic transcription factors (TFs) during vertebrate brain development. Zics are required for correct neurulation in mammals via a mechanism that is poorly understood. We have uncovered an essential role for zic2a and zic5 in hinge point (HP) formation during cranial neurulation in zebrafish. HP formation is an essential step in primary neurulation,

but has not been explored during secondary neurulation, typical of lower vertebrates. Intriguingly, mouse zic2 was recently shown to be involved in HP formation (Ybot-Gonzalez et al., 2007). To understand this conserved aspect of zic function, we are conducting experiments, including microarray hybridizations, aimed at identifying transcriptional targets of zic2a. Correct expression of zic genes is essential for their function, yet the mechanism of its regulation is not understood. We are using bioinformatics, site-directed mutagenesis and in vivo transgenic reporter assays to identify transcriptional regulators of zics. This approach has identified CDP/cux/cutl, conserved homeobox TFs, as essential novel controls of zic transcription. CDP/cux proteins regulate neural cell cycle progression in mammals, but have not been implicated in neurulation. We have previously shown that zic2 and zic5 mediate the mitogenic function of canonical Wnt signaling in the brain primordium. Collectively, our data suggest that zic genes are an important conserved node in the genetic regulatory network that coordinates cell cycle progression (growth vs differentiation) with morphogenesis in the developing neural tube.

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Program/Abstract # 161
Requirements for ovo orthologues in zebrafish neural tube and neural crest development
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The neural crest (NC) develops at the border between the non-neural and neural ectoderm of vertebrate embryos, and gives rise to a variety of different cell types such as cartilage and bone of the skull and pigment cells of the skin. In order to arrive at their final locations, NC cells delaminate from the ectoderm and migrate extensively throughout the body. Lineage tracing studies have shown that NC cell fates are specified prior to overt migration in zebrafish, but genetic evidence to support this idea has been limited. *Wnt* signaling promotes pigment cell fates at the expense of other NC-derivatives. Ovo transcription factors are known to respond to *Wnt* signaling to regulate several aspects of mammalian development. We have identified two zebrafish *ovo* orthologues, *ovo1* and *ovo2*, expressed in NC cells and in the neural tube. Injection of morpholino oligonucleotides targeting either *Ovo* causes defects in migration of pigment precursors but not other NC lineages. Inhibiting *Wnt* signaling reduces expression of *ovo1*, suggesting that *ovo* genes mediate *Wnt* functions in pigment specification as well as their migration. *Ovo* may regulate the progressive emergence of distinct subpopulations of NC cells from the dorsal neural tube. Consistent with this hypothesis, *Ovo* morphants have compromised neuroepithelial integrity and open neural tubes. Rhodamine-labeled wild type cells transplanted into the neural tube of *Ovo* morphants failed to cross the midline, suggesting that *Ovo* acts non-cell autonomously. One explanation for this comes from our finding that *Ovo* genetically interacts with *N-cadherin*, suggesting that it regulates neural tube and NC morphogenesis via modulation of cell adhesion.

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Program/Abstract # 162
The zebrafish dob/fgf20a mutant models human craniosynostotic syndromes with midfacial hypoplasia
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Craniosynostosis is the premature fusion of skull sutures, and in most of the more common craniosynostotic syndromes this condition is coupled with midfacial hypoplasia. Affected individuals may present restricted brain growth, cognitive dysfunction, and problems with hearing, speaking, eating, and breathing. While recent progress has been made in identifying the genetic basis of craniosynostosis and its associated syndromes, the diagnosis and treatment of these disorders is still complicated by their broad etiologies and variable expressivities. Developmental variation is therefore a considerable obstacle in craniosynostotic research. Using morphometric analyses, we were able to quantify the cranial shapes of *dob/fgf20a* mutant zebrafish and their wild-type siblings, remove the effects of developmental noise, and identify statistically significant mutational effects with a high degree of precision. We coupled this approach with whole-mount in situ hybridization studies of *fgf20a* expression in wild-type fish in order to compare the regions of anatomical distortion in mutants with regions of *fgf20a* expression in normal fish. Mutant fish exhibit aberrant suturing, distorted skull vault development, and midfacial hypoplasia that are strongly consistent with Apert's, Crouzon's, Pfeiffer's, and Saethre–Chotzen syndromes. The areas of skull deformation coincide with regions in which *fgf20a* is expressed in wild-type specimens. Our results offer the zebrafish *dob/fgf20a* mutant as a new experimentally tractable model with which to examine the developmental pathology of these diseases.

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Program/Abstract # 163
Craniofacial phenotypes of the knypek (glypican4) mutant zebrafish
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The Wnt/PCP pathway controls cell behavior by regulating cell polarity and the cytoskeleton. Glypicans, a class of membrane-linked heparan sulfate proteoglycans, interact with secreted ligands, including Wnts, to modulate their signaling potential. The zebrafish head skeleton is ideally suited for analysis of chondrocyte behavior and skull morphogenesis downstream of this pathway. Here, we describe the craniofacial development of a zebrafish *knypek* mutant, which is disrupted in the gene coding for glypican4. *Kny/gpc4* homozygotes develop dramatically misshapen head cartilages, similar to malformations observed in the *Wnt5* mutant *pipe tail*. In the early larvae we observed a loss of chondrocyte polarity, failure of cell–cell intercalation, and consequent defects in the elongation of larval cartilage elements. Adult *kny/gpc4* mutant fish (>1 y) have persistently abnormal chondrocyte organization and significant malformations of mature cranial bones. Morphogenetic defects include a shortening of the rostral-most skull and frequent loss of the symplectic, an endochondral element in the zebrafish jaw suspension. Finally, we report the unexpected result that all *knypek* mutant adults lack barbels, paired dermal appendages normally found in zebrafish and many other cyprinid species. To our knowledge, *gpc-4* is the first gene to be linked to barbel outgrowth, which does not occur until the juvenile stage. This novel phenotype suggests that late-acting glypicans may be critical in the morphogenesis and repeated evolution of these fish sensory structures.

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